A spectrophotometric method for the determination of Mefenamic acid in pharmaceutical dosage form

Mohammed Habib Rashid*, Susan Wadie Sarsam**, Najib Al-Sabea***

* Ministry of Health
**Dept. of Pharm. Chem., College of Pharmacy, University of Baghdad
sarsam14@yahoo.com

Abstract:
A new, stable and accurate UV spectrophotometric method was introduced for the determination of Mefenamic acid content in pure pharmaceutical material and in tablet dosage form. The method was based on the formation of a metal complex between Mefenamic acid and Nickel (II), which showed to exhibit a maximum absorbance at 360 nm. The analytical results obtained for both the pure compound and the four samples from different pharmaceutical brands available in the Iraqi pharmaceutical market were validated statistically. A comparison of the results obtained for the brands under study has revealed that Mefenamic acid content of the different brands was in good agreement with the labeled values and was within the permitted allowed percentage limits according to the United States pharmacopeia.

Key words: Mefenamic acid; spectrophotometry; metal complex.

Introduction:
Mefenamic acid (MFA), 2-[(2, 3-dimethyl phenyl) amino] benzoic acid, is a non-steroidal anti-inflammatory drug (NSAID) which belongs to anthranilic acid class (Fig.1). It is used as analgesic and anti-inflammatory agent for the treatment of dental pain, headache, postoperative pain, osteoarthritis and dysmenorrhea.[1] It acts as a non-selective inhibitor of the enzyme cyclooxygenase, inhibiting both the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isoenzymes. Cyclooxygenase catalyzes the formation of prostaglandins and thromboxane from arachidonic acid (itself derived from the cellular phospholipid bilayer by phospholipase A2). Prostaglandins act as messenger molecules in the process of inflammation.[2-4]

Figure-1: Structure of Mefenamic acid.
of mefenamic acid. The USP adopted a high performance liquid chromatographic (HPLC) method for the estimation of MFA in raw materials and its dosage forms while an acid-base titration method for its analysis was described in the BP.[5,6] Different analytical methods have been developed for the quantitative estimation of MFA in pharmaceutical formulations and biological fluids.[7] Several simple spectrophotometric methods for the determination of MFA in different brands of MFA tablets have been reported.[1,8-10] A spectrophotometric method was developed for the determination of MFA in the pure material and in pharmaceutical dosage forms. The method is based on their complexation with copper (II) ammonium sulphate. Following extraction and treatment with diethyldithiocarbamate solution, another copper (II) complex (λmax 430 nm) was formed.[11,12] Another spectrophotometric method was described based on reaction of MFA with Cobalt (II) whereby a deep brown colored complex having maximum absorbance (λmax) at 560 nm was produced. [13] A colorimetric method for the quantitative determination of MFA in bulk and pharmaceutical dosage forms was used based on reaction of MFA being carboxylic compound with 2-nitrophenylhydrazine hydrochloride to give an intensive violet color at λmax 550 nm.[14] MFA content was estimated utilizing three other simple and rapid spectrophotometric methods.[7] The first method is based on the development of red colored product having λmax at 520 nm by the reaction of the N-donor MFA with the π-acceptor p-chloranilic acid. The second method recorded an oxidative reaction of MFA with N-bromosuccinamide, resulted in the development of a yellow colored product with λmax at 362 nm. The third method is based on the formation of an oxidative coupling product by the reaction of MFA with 3-methylbenzo-thiazolin-2-one hydrazone as a chromogenic reagent in presence of ferric chloride solution. A green color product shows peak at 602 nm was developed. Other spectrophotometric methods were described, based on the formation of a colored species with MBTH through oxidation by Ce (IV) or Fe (III). [15] A spectrofluorometric method was developed for determination of MFA in pharmaceutical preparation and human urine.[16] The method is based on the oxidation of MFA with cerium (IV) to give cerium (III). Atomic absorption spectrometric methods for the quantitative estimation of MFA were reported, these methods are based on the formation of metal complexes of MFA with cupric chloride or cobaltous chloride.[17] Our study aimed at finding a new, sensitive and accurate method for the spectrophotometric determination of MFA in pure pharmaceutical material and in solid dosage form. The spectrophotometric method depends on the formation of metal complex of MFA with Nickel chloride.

Experimental:
Chemicals and instruments
Mefenamic acid was supplied from Al-sharq al-awsat drug factory (Iraq). All the chemicals and solvents used were of Analytical grade. All spectral measurements were carried out on computerized UV visible Shimadzu 1800 double beam spectrophotometer.

UV spectrophotometric analysis of Mefenamic acid (MFA)
2 mg /ml MFA solution in DCM was prepared. The solution was scanned between 200-800 nm against DCM as a blank using UV-visible spectrophotometer.

Stock solution preparation
To a 50 ml volumetric flask was added an accurately weighted 100 mg of pure MFA, dissolved in DCM and the volume was made up to the mark. Then the solution was transferred to a beaker and 5 ml of 1% NiCl2 solution was added, followed by the addition of 10 ml 0.1 M HNO3 for
digestion with vigorous shaking using magnetic stirrer for 15 min. A turbid pale yellow solution is produced after the addition of HNO3, then the solvent was evaporated to yield a solid powder. To the solid powder distilled water and DCM were added, transferred to a separating funnel, the DCM layer which contains MFA-Ni complex was separated, transferred to 50 ml volumetric flask and the volume was completed up to the mark. The solution was scanned in the region between 200-800 nm against a blank solution of DCM.

**Preparation of standard curve**
Multiple dilutions were made from the stock solution by transferring (0.4, 0.8, 1.2, 1.6, 2, 2.4, 2.8 ml) from 2 mg/ml stock solution of MFA complex into a series of 10 ml volumetric flasks and the volume was made up to the mark with DCM. The concentrations of MFA solutions obtained were (0.08, 0.16, 0.24, 0.32, 0.4, 0.48, and 0.56 mg/ml).

**Preparation and analysis of tablet samples**
Twenty tablets of each of the four different brands (mefril, piostan, painex, fenam) were accurately weighed and ground into fine powder separately. An amount of the powder equivalent to 100 mg from each brand sample was accurately weighed, shaken with (3×30 ml) methanol, filtered and washed. Then 5 ml of 1% Nickel chloride solution was added, followed by the addition of 10 ml 0.1 M nitric acid (for digestion), a turbid pale yellow solution was produced after vigorous shaking for 15 minutes. The solvent was evaporated to yield a solid powder. The solid powder was transferred to a separatory funnel with 100 ml distilled water and extracted with (3×30 ml) DCM. The extracts were collected to a volumetric flask (100 ml) and the volume was completed with DCM.

**Preparation of 1% nickel chloride (NiCl2) solution**
1% solution of Nickel chloride was prepared from NiCl2.6H2O in distilled water. The solution was scanned between 200-800 nm using UV-visible spectrophotometer and was found to exhibit an absorption maximum at 394 nm (Fig.2).

![Figure 2: Absorption spectrum of NiCl2 in distilled water](image)

**Checking the method in different experimental conditions**

**Temperature**
Two temperatures were selected to check the stability of the produced complex.

Stock solutions, prepared by complexation of MFA with Ni (II), were heated on a water bath for 10 min. at two different temps. 40 and 50° C. Solutions were cooled and scanned.
Time
After formation of complex, stock solution prepared was scanned at different time intervals: (0, 10, 20, 30, 40, 50 and 60) minutes and (7, 14, 21 and 30) days.

Results and Discussion:
The absorption spectrum of standard MFA in DCM illustrated the existence of two absorption maxima at 280 nm and 348 nm respectively as shown in Figure 3. The obtained absorption spectrum was analogous to that reported for MFA in ethyl acetate which showed two absorption maxima at 280 and 350 nm. [18]

![Absorption spectrum of Mefenamic acid in DCM](image1)

**Figure -3:** Absorption spectrum of Mefenamic acid in DCM

The reaction of MFA in DCM with NiCl₂ resulted in the formation of a metal complex between MFA and Nickel (II). The absorption spectrum of MFA-Ni (II) complex revealed a shift in the wavelength with an optimal peak (λ_{max}) at 360 nm (Fig-4). The shift in λ_{max} towards longer wavelength is an indication of a complex formation.

![Absorption spectrum of Mefenamic acid complex with Nickel (II)](image2)

**Figure -4:** Absorption spectrum of Mefenamic acid complex with Nickel (II)

The standard curve of MFA complex was constructed by plotting the concentrations of the standard solutions versus their corresponding absorbance obtained at 360 nm as shown in Fig.5.
A linear calibration curve was obtained which indicates that this method has good linearity and it obeys Beer’s Lambert law within the concentration range of 0.08-0.56 mg/ml of MFA with a correlation coefficient of 0.9976. The straight line equation is given by:

\[ y = 0.0997 + 0.1277x \]

Where \( y \) is the absorbance and \( x \) is the concentration of MFA solutions.

Table 1 represents different brands of MFA tablets used in the assay.

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Company</th>
<th>Country of origin</th>
<th>Labeled content (mg)</th>
<th>Weight of single tablet (mg)</th>
<th>Weight of 20 tablets (mg)</th>
<th>Corresponding to 100 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mefril</td>
<td>Micro</td>
<td>India</td>
<td>500</td>
<td>850</td>
<td>17000</td>
<td>170 mg</td>
</tr>
<tr>
<td>Piostan</td>
<td>Pioneer</td>
<td>Iraq</td>
<td>500</td>
<td>720</td>
<td>14400</td>
<td>144 mg</td>
</tr>
<tr>
<td>Painex</td>
<td>Joswe</td>
<td>Jordan</td>
<td>500</td>
<td>700</td>
<td>14000</td>
<td>140 mg</td>
</tr>
<tr>
<td>Fenam</td>
<td>JPI</td>
<td>Jordan</td>
<td>500</td>
<td>750</td>
<td>15000</td>
<td>150 mg</td>
</tr>
</tbody>
</table>

The amount of MFA present in each sample of different brand used in assay, was calculated by using the straight line equation:

\[ y = 0.0997 + 0.1277x \]

The standard deviation (SD), percentage of relative standard deviation (%RSD), percent of recovery (% recovery) and percent of error (% error) were represented in table-2.
Table-2: The determination of Mefenamic acid in the tested samples by the UV method.

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Labeled content (mg)</th>
<th>Calculated content (mg)</th>
<th>SD</th>
<th>% RSD</th>
<th>% Recovery</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mefril</td>
<td>500</td>
<td>517</td>
<td>3.37</td>
<td>0.39</td>
<td>103.4</td>
<td>3.4</td>
</tr>
<tr>
<td>Piostan</td>
<td>500</td>
<td>510</td>
<td>2.24</td>
<td>0.311</td>
<td>102</td>
<td>2</td>
</tr>
<tr>
<td>Painex</td>
<td>500</td>
<td>490</td>
<td>2.19</td>
<td>0.312</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>Fenam</td>
<td>500</td>
<td>482</td>
<td>1.15</td>
<td>0.15</td>
<td>96.4</td>
<td>3.6</td>
</tr>
</tbody>
</table>

The mean at specific confidence limit of 95% for the MFA samples was shown in Table 3:

Table-3: Mean at confidence limit of 95 % level.

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Labeled content (mg)</th>
<th>Calculated content (mg)</th>
<th>Mean at Confidence limit 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mefril</td>
<td>500</td>
<td>517</td>
<td>517±1.5</td>
</tr>
<tr>
<td>Piostan</td>
<td>500</td>
<td>510</td>
<td>510±0.99</td>
</tr>
<tr>
<td>Painex</td>
<td>500</td>
<td>490</td>
<td>490±0.97</td>
</tr>
<tr>
<td>Fenam</td>
<td>500</td>
<td>482</td>
<td>482±0.51</td>
</tr>
</tbody>
</table>

From the results obtained in Tables 2 and 3, it is clearly shown that the developed UV method gave good recovery values in agreement with the labeled amounts for each the tested samples collected from different pharmaceutical companies. Additionally, these values were within the permitted limit stated by the USP (90-110 % of the labeled amount of MFA).\[5\]

The effect of different experimental conditions on the stability of the complex formed by the new UV spectrophotometric method was studied. No change was observed in the absorption spectrum of MFA-Ni (II) complex checked at different temperatures and different time intervals. The absorption spectrum still found to exhibit an absorption maxima $\lambda_{\text{max}}$ at 360 nm, irrespective of the exposure to different experimental conditions.

**Conclusion:**
A new stable and accurate UV spectrophotometric method was successfully developed for the quantitative determination of Mefenamic acid in pure material and in different brands of commercially available Mefenamic acid tablet formulation. The developed method was based on the formation of a complex between MFA and Ni (II).

Analysis of the sample obtained from different pharmaceutical brands revealed that they were in close agreement with
the labeled amounts and were within the limits stated by the USP.

References: